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TARGETING AMPK TO TREAT TYPE 2 DIABETES

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The AMP-activated protein kinase (AMPK) is an energy sensor that monitors AMP:ATP and ADP:ATP ratios in living cells (1). Once activated by rises in these ratios (signifying a fall in cellular energy), it acts to restore energy balance by switching on metabolic pathways that generate ATP (*catabolism*), while switching off processes that consume ATP, including synthesis and storage of macromolecules (*anabolism*). Not surprisingly, energy balance at the cellular and whole body levels are intimately connected. Obesity, which occurs when whole body energy intake exceeds energy expenditure for prolonged periods, is a major public health issue because it increases the risk of disorders such as Type 2 diabetes. In obese individuals, liver and muscle may store excess fat, leading to resistance to the hormone insulin. Released when blood glucose rises after meals, insulin normally promotes glucose uptake by muscle and represses glucose production by liver, thus rapidly returning blood glucose to normal. This is impaired in insulin-resistant individuals and glucose may become persistently elevated, eventually causing debilitating or life-threatening complications. Because AMPK was known to promote muscle glucose uptake by insulin-independent mechanisms, it was proposed in 1999 that AMPK-activating drugs might represent a novel approach to treat diabetes (2). A report in this issue of Science (3), and another recent paper (4), represent the culmination of over 15 years of development of this concept by pharmaceutical companies.

The role of AMPK in diabetes treatment was reinforced in 2001 by findings that it was activated by metformin (5). Introduced in the 1950s, metformin remains the front-line drug treatment in Type 2 diabetes, prescribed to >150,000,000 patients worldwide. It activates AMPK by inhibiting mitochondrial ATP synthesis, thus increasing cellular AMP/ADP. Given this indirect mechanism, it is not surprising that metformin has multiple effects, some (6) but not all (7) being AMPK-independent. Nevertheless, because of its pharmacokinetics and cellular uptake mechanisms, effects of metformin are largely confined to liver and gut, so drugs that act on muscle might have additional benefits.

AMPK exists as complexes of three subunits, a catalytic α and regulatory β and γ subunits. Each occurs as multiple isoforms ($\alpha 1/\alpha 2$, $\beta 1/\beta 2$, $\gamma 1/\gamma 2/\gamma 3$), generating up to twelve possible combinations (1). Repeated sequences in the γ subunits generate three binding sites for the regulatory nucleotides AMP, ADP and ATP, and some AMPK activators (e.g. AICAR) target these. However, a new regulatory mechanism was discovered in 2006 when a novel activator, A-769662, was identified in a

high-throughput screen (8). Although mimicking two of the activating effects of AMP on AMPK, A-769662 clearly bound at a different site (9), which was identified when an AMPK complex was crystallized in the presence of A-769662 or another activator, 991. The structures revealed that A769662 and 991 bind in a tunnel between two domains of the α and β subunits (Fig. 1A) (10). This site is unique to AMPK and has been termed the Allosteric Drug and Metabolite (ADaM) site (11) because, although currently only known to bind synthetic activators, it is widely assumed there are unidentified metabolite(s) that bind there (one natural product, salicylate, binds the site (12), but only occurs naturally in plants).

Fig. 1B/C shows structures of ADaM site activators, including the new compound reported in Science by Merck (MK-8722 (3)), three from Pfizer (PF-249, PF-06409577, PF-739 (4, 13)) and one from Mercury Pharmaceuticals (MT 63-78 (14)). Those in 1B only activate AMPK complexes containing the β 1 isoform, while those in 1C also activate β 2 complexes. This is important because liver expresses mainly β 1 (at least in rodents) while muscle expresses mainly β 2. MK-8722 activates all twelve possible human AMPK complexes, with EC_{50} values ranging from 1-6 nM for β 1 and 30-60 nM for β 2 complexes. It also enhanced glucose uptake by skeletal muscle in vitro, and improved glucose tolerance in several rodent models of diabetes in vivo, while in non-human primates (rhesus macaques) with Type 2 diabetes, it improved glucose tolerance and lowered HbA1c, a marker of persistently elevated blood glucose (3).

The Pfizer compound PF-739 is structurally very similar to MK-8722 (Fig. 1C). A comparison of PF-739 (pan- β) with PF-249 (β 1-selective) showed that only the former activated muscle AMPK and lowered blood glucose in mice with diet-induced obesity (4). Neither compound affected glucose production by the liver, while only PF-739 increased peripheral glucose disposal in vivo. Consistent with this, a liver-specific double knockout of AMPK (α 1^{-/-} α 2^{-/-}) had no impact on glucose reduction by PF-739, while a muscle-specific double knockout attenuated it. PF-739 also lowered blood glucose and insulin in non-diabetic Cynomolgus monkeys. While reducing blood glucose therefore requires a pan- β activator, β 1-selective activators may be useful for other purposes. For example, PF-06409577 activated AMPK in the kidney, and reduced urinary protein and other markers of kidney damage in a rat model of diabetic kidney disease (13).

While these results seem very promising, concerns about adverse effects of AMPK activators were raised by previous studies of humans with dominantly-acting mutations in the *PRKAG2* gene, encoding the AMPK- γ 2 subunit. These mutations increase basal AMPK activity, and were associated with increases in cardiac glycogen content and hypertrophy, as well as potentially life-threatening cardiac arrhythmias (15). In rats and rhesus macaques, long-term use of MK-8722 was indeed associated with increased cardiac glycogen content and hypertrophy, although not with arrhythmias which may be a consequence of elevated AMPK during fetal development (3). Effects of the Pfizer compounds on these cardiac parameters were not reported, so it remains unclear whether these effects on the heart are common to all pan- β activators.

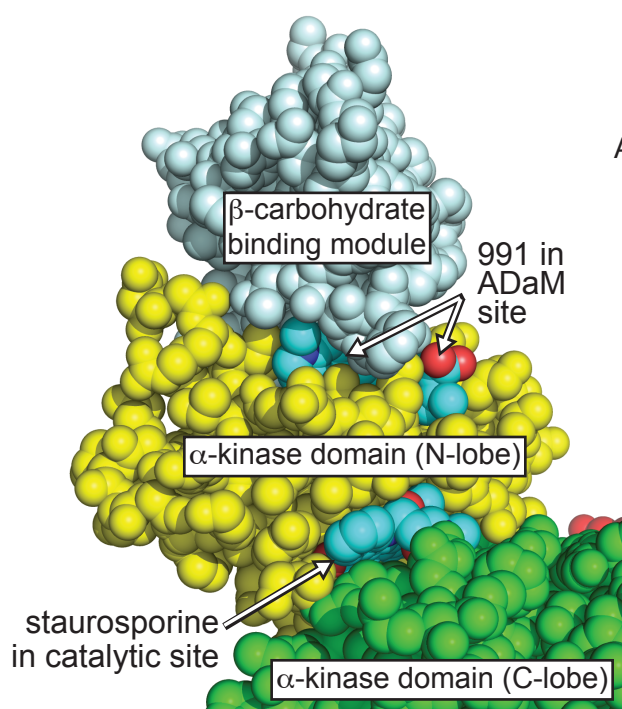
In summary, the potent and very similar pan- β activators MK-8722 and PF-739 were very effective in reversing elevated blood glucose in rodents and non-human primates, and this was due to activation of AMPK in muscle, not liver. This might make them valuable adjuncts to metformin, which acts primarily on the liver. One caveat is their potential to cause cardiac hypertrophy, which is likely to raise concerns with regulatory authorities. However, as the authors point out (3), cardiac hypertrophy observed with MK-8722 is reminiscent of that found in elite athletes (which may even be the result of AMPK activation during training), and does not necessarily have adverse consequences. Finally, although there are now seven AMPK activators that bind at the ADaM site, whether there is a natural metabolite in mammalian cells that binds there remains unclear, although those in the field may now be stimulated to look.

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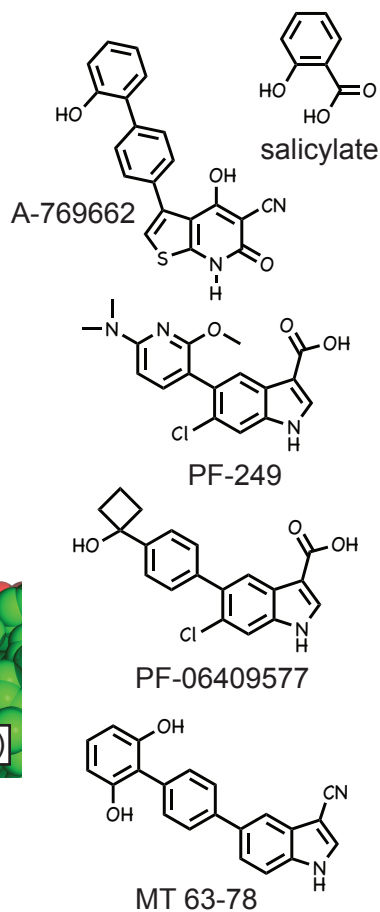
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Figure 1: (A) Location of ADaM and catalytic sites in AMPK; (B) structures of β 1-selective activators; and (C) structures of pan- β activators. The view in (A) was rendered using MacPyMOL v1.7.4.2 using the atomic co-ordinates of the human α 2 β 1 γ 1 complex (PDB ID 4CFE (10)); the model is in sphere view with H atoms omitted. Staurosporine and 991 are shown with C atoms pale blue and O atoms red. The ADaM site is a tunnel between the β subunit carbohydrate-binding module and the N-lobe of the α subunit kinase domain, so that only the ends of the 991 molecule are visible. The kinase inhibitor staurosporine is bound in the active site between the N- and C-lobes of the kinase domain, where the substrate MgATP would bind.

A) Location of ADaM and catalytic sites



B) β 1-selective activators:



C) pan- β activators:

